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Acid Formation Potential in Mineral Mine Spoils and Overburden: Bioassay Evaluation

COOP agreement - UT State Univ

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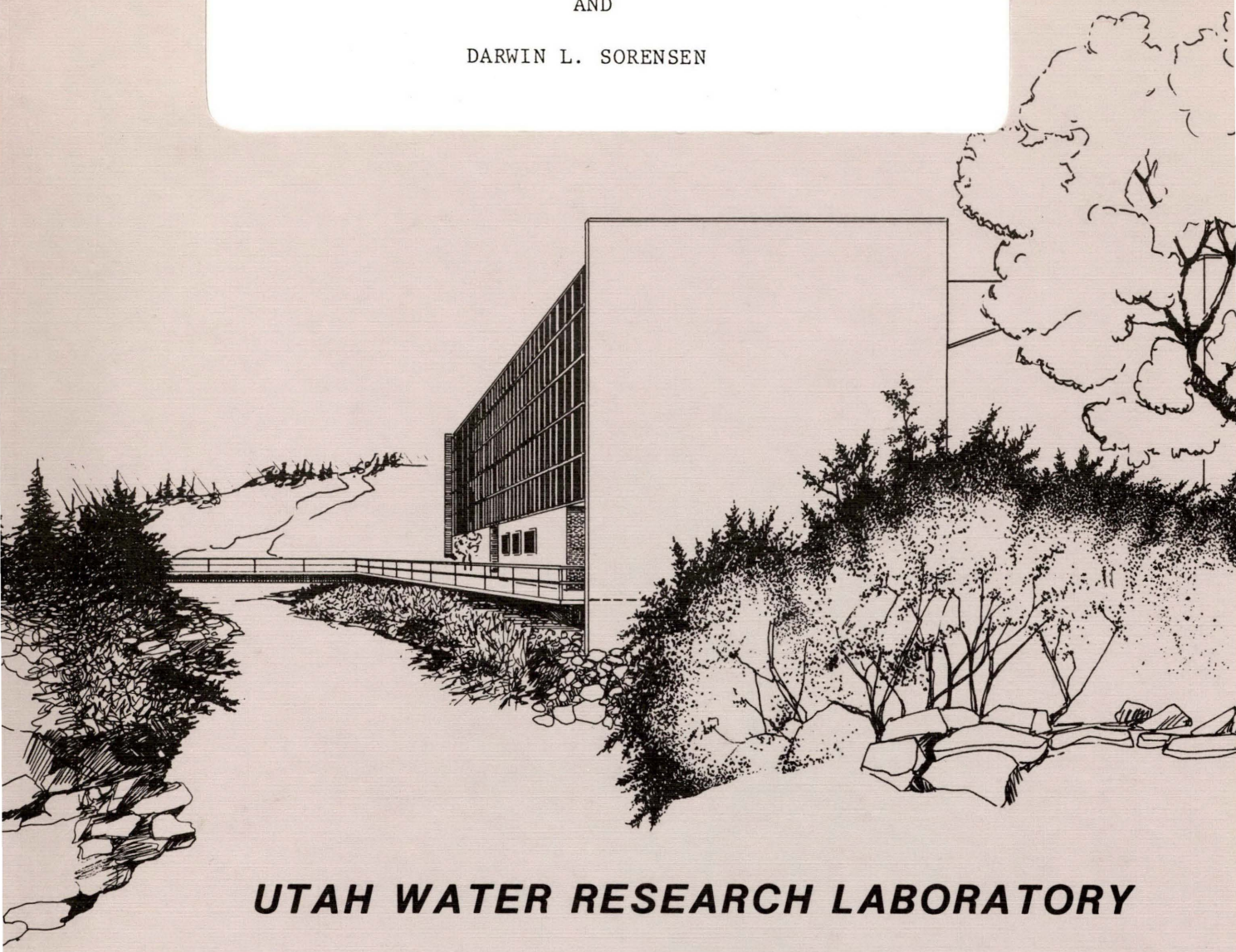

UTAH WATER RESEARCH LABORATORY

College of Engineering
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Logan, Utah

FINAL REPORT FOR COOPERATIVE AGREEMENT 22-C-4-INT-120
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INTRODUCTION

Mineral mine spoils frequently contain pyritic minerals and native sulfur that are subject to oxidation to sulfuric acid in sufficient quantities to cause acidification. Serious environmental damage results as acid, solubilized toxic metals, and salinity move from these acidic materials into streams, groundwater, and dust. Predicting acid formation in mine wastes and controlling the acidification process are major challenges facing mine land reclamation practitioners across the nation.

The actual oxidation of pyritic minerals in mine spoils is complex, and no single reaction stoichiometry is adequate to explain the amount of acid produced from reduced sulfur (Bruynesteyn and Hackl 1982). However, with a few simplifying assumptions about the mineral forms of reduced sulfur in mine spoils and the stoichoimetry of the oxidation reactions, it is easy to estimate the amount of acid that would be produced by the complete oxidation and hydrolysis of the mineral (Bruynesteyn and Hackl 1982, Sorensen et al. 1980). If the acid neutralization capacity of the spoil material is also estimated and then subtracted from the acid production potential estimated from the sulfur content of the spoil, a "chemical" estimate of the net acid production potential of the spoil can be made (Duncan and Walden 1975). This is a conservative approach and does not incorporate the effects of bacteriological oxidation of sulfur. Although sulfide minerals will oxidize upon exposure to atmospheric oxygen, the process is accelerated many thousandfold by the metabolic activities of the Thiobacillus group of bacteria (Harvard University 1970, Ohio State University 1970). In essence, acidification of mine spoils would be nil without the activity of the Thiobacilli.

Chemical tests do not incorporate the effects of form, dispersion, and exposure which regulate the availability of the sulfur and sulfide to the Thiobacilli. Factors which may limit the activity of Thiobacilli (toxins, pH, nutrient availability, etc.) are also ignored when a bioassay for acid production is not included in the analysis.

Duncan and Walden (1975) developed a procedure which incorporates both chemical estimation of net acid production and a bioassay system. In their procedure, if a sample of mine spoil or overburden lacks sufficient neutralizing capacity to control the acid production estimated from a total sulfur determination, it is subjected to a closed culture bioassay in which slurried spoil material is inoculated with Thiobacillus ferrooxidans. The spoil or overburden material is a potential acid producer if the maximum amount of acid produced in the bioassay flask exceeds the neutralizing capacity of the weight of spoil material that was originally added to the flask. In bioassays (McLean and Sorensen 1983) of 22 mine tailings samples from the western United States that were chemically determined to be net acid producers, only 11 displayed acid production using a modified Duncan and Walden (1975) bioassay procedure.

Bruynesteyn and Hackl (1982) reviewed the application of the Duncan and Walden (1975) procedure, and recognized that the closed culture bioassay system does not simulate the open, flushing type system that is most common in nature. They recommend that a larger scale leaching column system using periodic, once-through leaching with distilled water be used to ultimately determine the acid producing potential of overburden or spoil. The accumulation of acid and other toxic metabolic products

along with limited oxygen availability may limit the activity of the Thiobacilli in the closed bioassay flask.

The process of sulfur oxidation within a mineral matrix may be compared to the mineralization and oxidation of nitrogen from soil organic matter. Both processes are microbially mediated, are most rapid under aerobic condition, and the products of the processes are removed from the site with soil water. Stanford and Smith (1972) developed a method for estimating the nitrogen mineralization potential of a soil which uses periodic leaching of a 15 g sample of soil mixed with an equal weight of sand or vermiculite. After each leaching event mineral nutrients are replaced, and the concentrations of soluble nitrogen compounds in the leachate are measured. For many soils, the total mineralizable nitrogen can be predicted from the amount of nitrogen solubilized after 2 weeks of leaching (Stanford et al. 1974). This same general concept can possibly be applied in predicting the amount of acid produced from finely ground mine spoil or overburden.

The catalytic activity of the sulfur and iron oxidizing bacteria in any bioassay system may be enhanced by creating a more favorable environment for their growth. Thiobacillus ferrooxidans and Thiobacillus thiooxidans have optimum pH ranges of 2.5 to 5.8 and 2.0 to 3.5 respectively (Vishniac 1974). A ssuring the acidic pH of the spoil material by acidification of the nutrient medium might enhance the activity of these organisms (Duncan and Walden 1975, Bruynesteyn and Hackl 1982). Recent research (Wakao et al. 1983) has shown that small amounts of non-ionic surfactant greatly accelerate the oxidation of pyrite by T. ferrooxidans. Inclusion of a non-ionic surfactant in a bioassay may enhance sensitivity

and shorten the time necessary to estimate the acid producing potential of mineral material.

In nature the oxidation of pyrite minerals, native sulfur, and other reduced sulfur compounds may contribute to acid production. Different species of Thiobacillus appear to be more adept at oxidation of different minerals. For example, T. thiooxidans is recognized for its ability to oxidize elemental sulfur (Vishniac 1974). Different species of Thiobacillus also have different pH optima for growth, and it is possible that a succession in species of Thiobacillus occurs as the environment becomes increasingly acidic. One possibility is that T. neopolitanus helps initiate the acidification process since it grows well at near neutral pH and oxidizes sulfur or partially reduced sulfur compounds such as thiosulfate (Vishniac 1974). Once the pH has dropped below the range for T. neapolitanus, T. thiooxidans and T. ferrooxidans may become active and increase the acidity to pH 2 or less. Inoculation of a bioassay with a mixed culture may enhance the sensitivity and speed of the assay by taking advantage of the special capabilities and succession of these organisms.

The objective of the present study was to begin the development of a bioassay for determining the acid formation potential in mineral mine spoils and overburden. We approached this problem by comparing the sensitivity of a closed culture assay with that of a leaching assay. The effects of surfactant addition, nutrient medium acidification, and inoculation with mixed species of Thiobacilli were evaluated.

MATERIALS AND METHODS

Mine spoils

Ten samples of mine spoil materials were collected by U.S. Forest Service personnel from seven mine sites in the Western United States: Two from the Blackbird copper-cobalt mine near Salmon, Idaho; one from a nickel mining site in the Six Rivers National Forest near Gasquet, California; one each from the McLaren and Glenngary mines near Cook City, Montana; one from the Solar Silver tailings pond near Helena, Montana; one from the Preachers Cove mill tailings near Clayton, Idaho, and three from the Leviathan mine near Reno, Nevada.

Chemical analyses

Samples were air dried and ground to pass a 100 mesh seive (0.15 mm nominal opening) and the pH, acid neutralization potential, and total sulfur content were determined. pH was measured in a stirred 50 percent (w/v) slurry of soil in water using a glass electrode and pH meter. Acid neutralization potential (alkalinity) was measured by titrating 10 g of sample with 0.25 N H_2SO_4 to pH 3.5, and was reported as mass of $CaCO_3$ per 100 g (Duncan and Walden 1975). Total sulfur content was determined from a Na_2CO_3-Na_2O_2 fusion of a 1 g sample in a nickel crucible (AOAC 1955) with sulfur in the resulting solution determined by Inductively Coupled Argon Plasma Spectroscopy (ICAP).

Oxidized sulfur (primarily sulfate) was extracted from selected samples using 2:3 concentrated HCl: deionized water (approx. 4.8M HCl). Five grams of sample were suspended in 50 ml of 2:3 HCl in a 125 ml

Erlenmeyer flask and shaken on an orbital shaker at 200 rpm for 30 minutes at room temperature. The slurry was then filtered through Whatman 40 filter paper, the flask was rinsed, and the material retained on the filter was leached with three or four small volumes of 2:3 HCl totaling 50 ml. The filtrate was diluted with deionized water to 250 ml and analyzed for sulfur by ICAP.

Non-HCl-extractable sulfur is the difference between total sulfur and HCl-extractable sulfur. Soluble and exchangeable sulfate was extracted using a solution of 0.15 percent $CaCl_2$ with 500 mg PO_4/l (Tabatabai 1982).

Acid forming potential of the mine spoil materials was calculated from total sulfur and non-extractable sulfur assuming a 1:1 molar ratio between sulfur and H_2SO_4 formed. Results are reported as equivalent weights of $CaCO_3$/100 g of spoil (Duncan and Walden 1975). Samples for bioassay were selected from those showing a net acid producing potential and were ball milled (wet) to pass a 400 mesh (0.038 mm nominal opening) seive.

Closed culture bioassay

The slurry flask bioassay test was performed as follows: Duplicate 30 g portions of sample (400 mesh) were placed in 250 ml Erlenmeyer flasks with 70 ml of nutrient solution containing 3 g $(NH_4)_2SO_4$, 0.1g KCl, 0.50 g K_2HPO_4, 0.50 g $MgSO_4 \cdot 7H_2O$, and 0.01g $Ca(NO_3)_2$ per liter. The effects of adjusting the nutrient solution pH to 3.5 and the addition of 0.001 percent Tween 20 were evaluated singly and in combination resulting in four treatments for each sample tested. The flasks were shaken for 4 hours, and the pH was monitored. Acid was added as

necessary to pH adjusted treatments to maintain the pH at 3.5. Samples were then inoculated with 3 ml of active Thiobacillus ferrooxidans culture. Flasks were covered with aluminum foil and placed on a orbital shaker at 100 rpm. Samples were incubated at room temperture (average: 21.5 C, range: 14.5-27 C).

To determine the amount of acid added initially, half of the sample (by weight) was removed from each flask after 1 hour incubation and was titrated to its original pH using 0.25 M NaOH.

The pH of the material in the bioassay flasks was monitored daily for 3 days, and then every third day. Microbial activity was assumed to have ceased when the pH remained constant. Samples were terminated by titrating the material to its original pH with 0.25 M NaOH. The amount of acid produced by microbial activity was calculated from the difference in the acid neutralized in the final titration and the amount of acid initially added to the system.

Leaching bioassay

The leaching bioassay was designed after a Stanford et al. (1974) procedure for estimating the potential mineralization of soil nitrogen. A mixture of 15 g mine spoils (400 mesh) and 15 g hydrochloric acid-rinsed white sand was placed into duplicate plastic cups (5.8 cm I.D. with three 4 mm drainage holes). Available acid was leached from the columns using 150 ml of 0.01 M $CaCL_2$. Nutrient solution containing 0.001 percent Tween 20 (treatments 2, 4, 6, and 8), sufficient H_2SO_4 to adjust pH to 3.5 (treatments 1, 2, 5, and 6), T. ferrooxidans (treatment 1-4), mixed culture of T. ferrooxidans, T. ferrooxidans and T. Thiooxidans

(treatments 5-8) was added to appropriate columns (Table 1.). The leachate collected was titrated with 0.25 M NaOH to pH 7.0. Samples were incubated at room temperature and leached weekly for 6 weeks with 150 ml 0.01 M $CaCl_2$, followed by 15 ml of the appropriately treated nutrient solution. No additional bacteria were added. Acid production was determined by titrating the leachate with 0.25 M NaOH to pH 6.0.

RESULTS AND DISCUSSION

Chemical estimation of acid producing potential

Results of total sulfur, HCl extractable sulfur, and soluble and exchangeable sulfur analyses are shown in Table 2 along with the acid producing potential calculated from total and non-HCl-extractable sulfur. With the exception of the Preachers Cove and Leviathan 6 samples, which were pH 8.3, all samples showed significant potential for acid formation based on total sulfur content. However, more than 30 percent of the total sulfur was extractable with 2:3 hydrochloric acid in all samples extracted except the Six Rivers sample. More than 90 percent of the total sulfur was HCl extractable in the McLaren and Solar Silver samples. In contrast, soluble and exchangeable sulfur was less than 32 percent of total sulfur in all samples.

Since the soluble and extractable sulfur in soils is mostly sulfate, and sulfide and elemental sulfur are not soluble in HCl, we assume that the difference between soluble and exchangeable and HCl extractable sulfur comes from insoluble basic iron or aluminum sulfate minerals such as jarosite ($KFe_3(OH)_6(SO_4)_2$), coquinbite ($Fe_2(SO_4)_3 \cdot 5H_2O$), or basalunite ($Al_4(OH)_{10}SO_4 \cdot 5H_2O$) (Tabatabi 1982). Since more

Table 1. Treatments applied in the leaching bioassay.

Treatment Number	Description	
	Inoculum	Nutrient Medium
1	*T. ferrooxidans*	pH 3.5
2	*T. ferrooxidans*	pH 3.5 Tween 20
3	*T. ferrooxidans*	(No amendments)
4	*T. ferrooxidans*	Tween 20
5	*T. ferrooxidans* *T. thiooxidans* *T. neapolitanus*	pH 3.5
6	*T. ferrooxidans* *T. thiooxidans* *T. neapolitanus*	pH 3.5 Tween 20
7	*T. ferrooxidans* *T. thiooxidans* *T. neapolitanus*	(No amendments)
8	*T. ferrooxidans* *T. thiooxidans* *T. neapolitanus*	Tween 20

Table 2. Mine spoil samples, their pH, acid neutralization potential to pH 3.5, total sulfur, soluble and exchangeable sulfur, HCl extractable sulfur, and chemically estimated acid formation potential.

Sample	pH	Acid Neutralization Potential ($mgCaCO_3/100$ mg)	Total Sulfur (%)	Net Acid Formation Potential from total S($mgCaCO_3/100$ g)	Soluble and Exchangeable S (%)	HCl Extractable S (%)	Net Acid Formation Potential from Non-HCl-Extractable S($mgCaCO_3/100$ g)
Blackbird brown	4.0	50	0.54	1,630	0.03	0.30	730
Blackbird yellow	3.3	0	4.38	13,700	0.06	1.34	9,480
Six Rivers	4.5	74	1.62	4,980	0.13	0.11	4,666
McLaren	3.5	0	0.97	3,030	0.19	0.94	94
Glenngary	2.7	0	3.88	12,100	0.55	-	-
Solar Silver	3.4	0	0.60	1,870	0.19	0.56	160
Preachers Cove	8.3	3560	0.18	0	0.009	-	-
Leviathan 2	4.3	251	1.17	3,400	0.30	0.36	2,309
Leviathan 4	2.5	0	5.56	17,300	0.19	-	-
Leviathan 6	8.3	2030	0.04	0	0.005	-	-

than 70 percent of the total sulfur is sulfate (oxidized sulfur) in the McLaren and Solar Silver samples extensive weathering is indicated, and relatively little reduced sulfur remains to produce acid. More than half of the total sulfur in the Blackbird brown sample was extractable with HCl indicating significant oxidation of the sulfur in this material as well. Despite the high fraction of oxidized sulfur in these samples, some reduced sulfur does remain, and acid production may continue, although the mass of acid expected would be greatly reduced.

Large amounts of acid could be produced from the non-HCL-extractable sulfur in the Blackbird yellow, the Six Rivers, and the Leviathan 2 samples. It is noteworthy that in the Six Rivers sample, only about 7 percent of the total sulfur was extractable with HCl, while the Blackbird yellow and Leviathan 2 samples had about 31 percent of their total sulfur content extractable with HCl. The Six Rivers sample is from a much younger and presumably less weathered spoil material than any of the other samples included in this study.

Closed culture evaluation of acid producing potential

The Blackbird brown, Blackbird yellow, Six Rivers, McLaren, and Solar Silver samples were assayed for acid production using the closed culture technique. A small, but insignificant, amount of acid was produced in the Blackbird brown sample. Only the Blackbird yellow sample showed a significant net decrease in pH. The addition of 0.001 percent surfactant Tween 20 to the nutrient medium had no significant effect on the amount or kinetics of acid production in this material. The pH of the Blackbird yellow slurry decreased to values below 1.8 in an average time of 24

days, afterwhich no further decrease in pH was observed. Acid equivalent to 1565± 70 mg $CaCO_3$/100g had been produced from the Blackbird yellow spoil at the end of the assay; sixfold less than that estimated from non-HCl-extractable sulfur. Despite its relatively high chemically estimated acid producing potential, the Six Rivers sample failed to produce appreciable amounts of acid.

Leaching assay acid production potential

The leaching assay included the Blackbird brown, Blackbird yellow, Six Rivers, McLaren, Solar Silver, and Leviathan 2 samples. Only the Blackbird brown, Blackbird yellow and McLaren samples produced measurable amounts of acid over the approximately 6 weeks incubation period.

Cumulative acid production for the Blackbird brown sample over time is illustrated in Figures 1 and 2. Acid production in this sample was essentially nil after about 3 weeks incubation (fourth leaching event). Figure 3 shows that average acid production during the first week of incubation was lowest in the Blackbird brown sample. Cumulative acid production remained lowest in this sample through the 6 weeks of incubation (Figures 4 and 5). No significant effect of adding a mixed Thiobacilli inoculum versus a single species (T. ferrooxidans) inoculum was observed in the Blackbird brown sample (Figures 4 and 5). Apparently, sulfur available for acid formation in this sample was quickly exhausted under the assay conditions.

Acid production in the McLaren sample was more vigorus and continued over the 6 week incubation period (Figures 6 and 7). Average acid production from this sample during the first week of incubation was

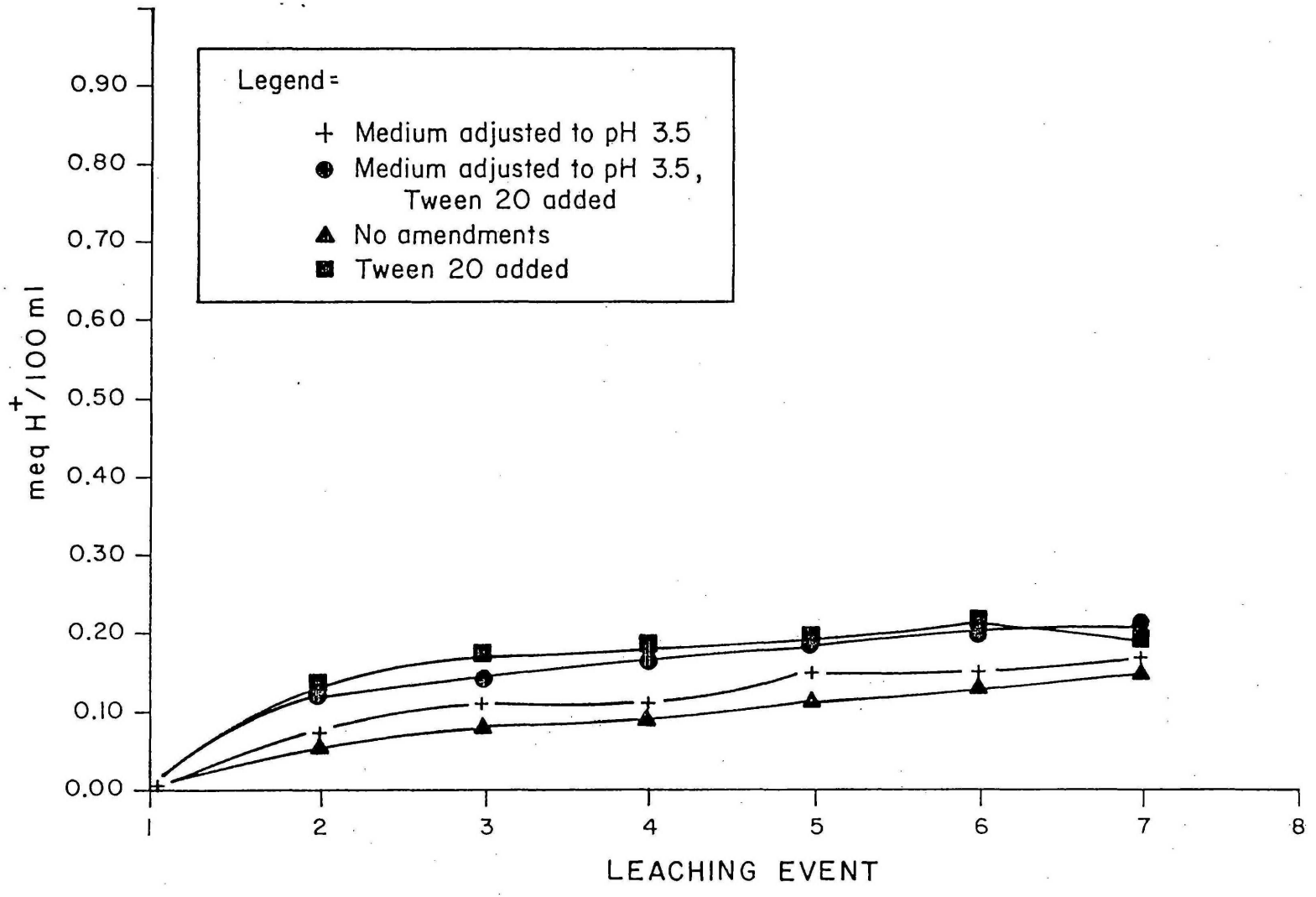

Figure 1. Cummulative acid production from the Blackbird brown spoil inoculated with T. ferrooxidans alone.

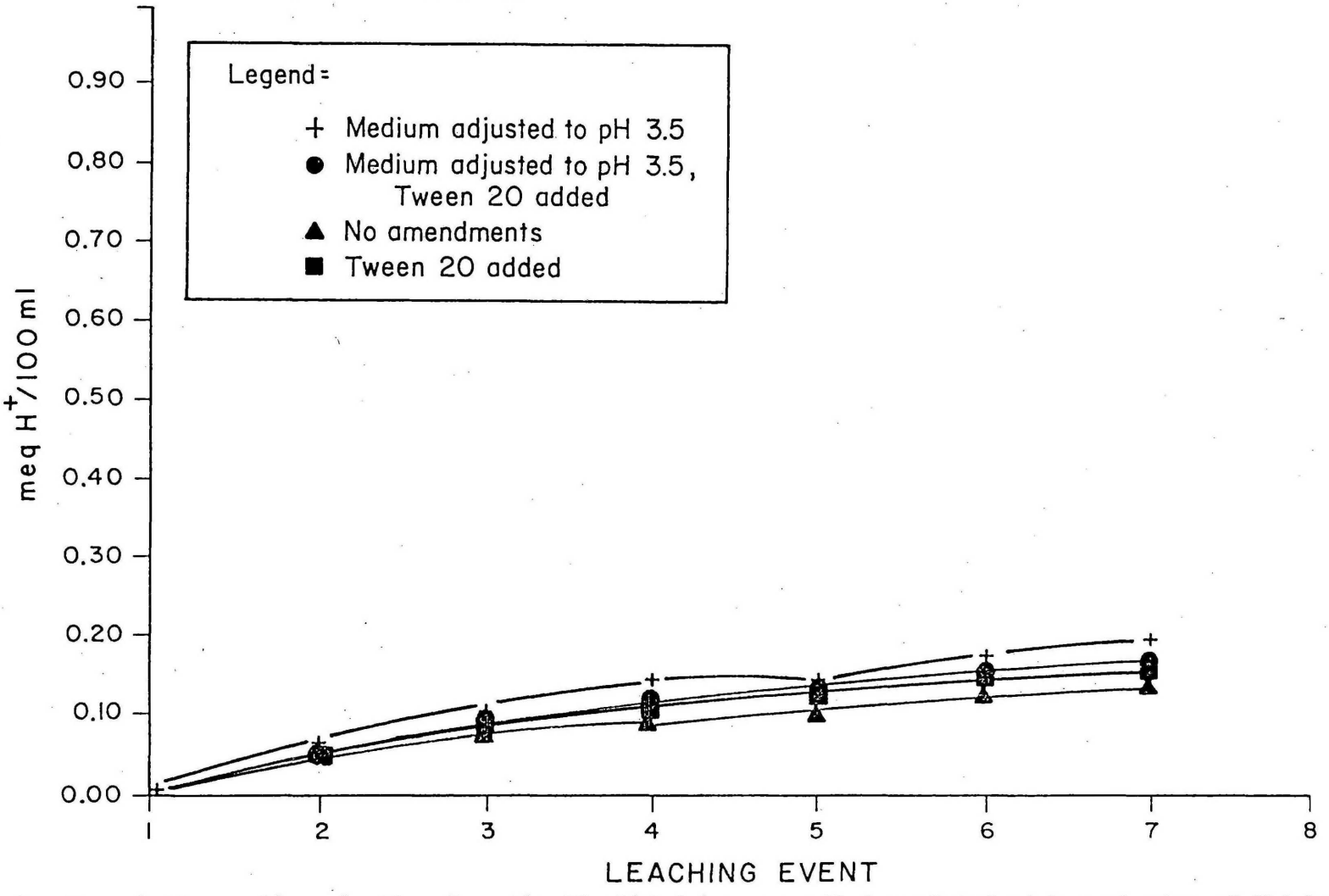

Figure 2. Cummulative acid production from the Blackbird brown spoil inoculated with a mixture of Thiobacilli.

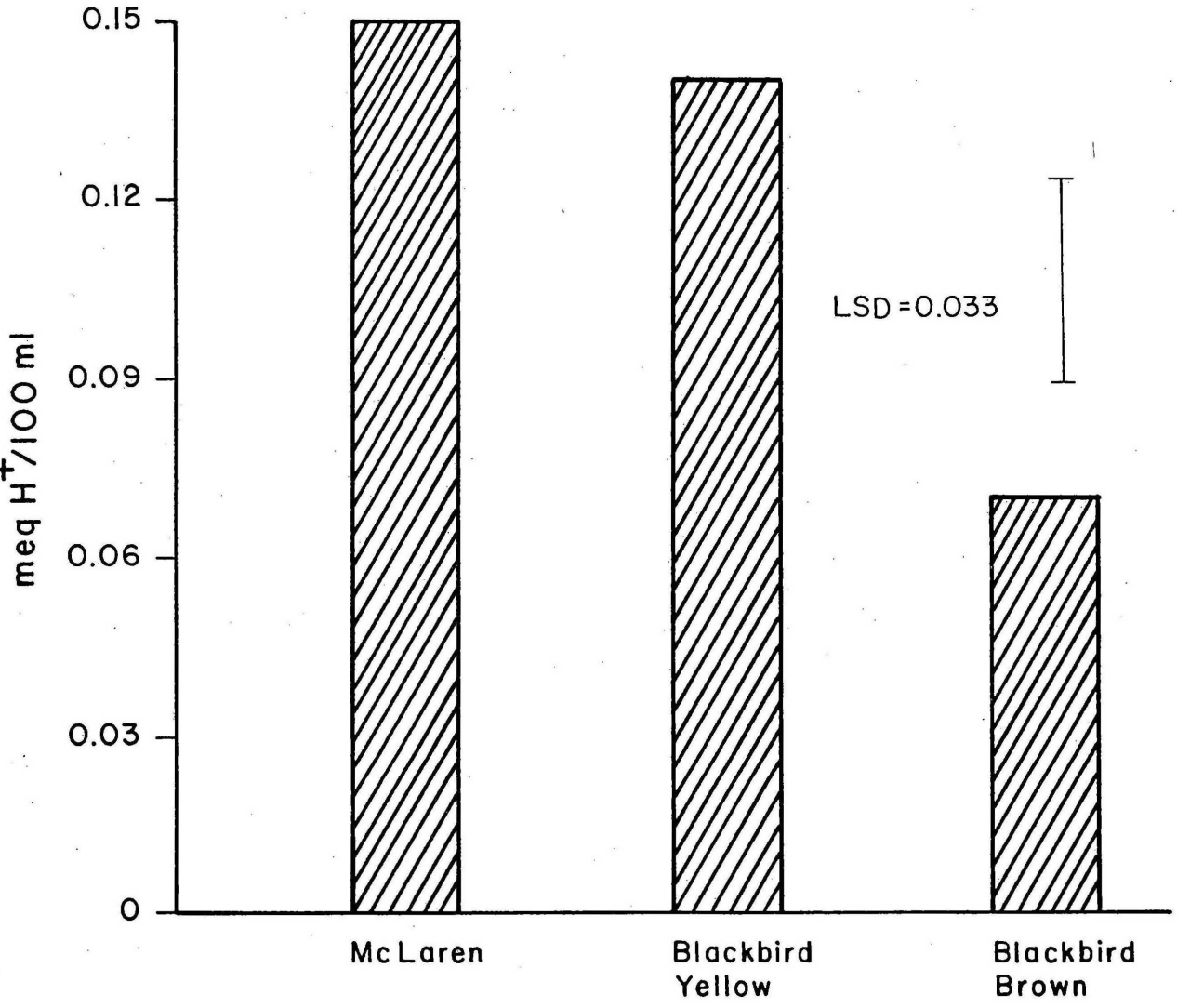

Figure 3. Acid production in the McLaren, Blackbird yellow, and Blackbird brown spoils after 1 week incubation. No significant effects of treatment were observed.

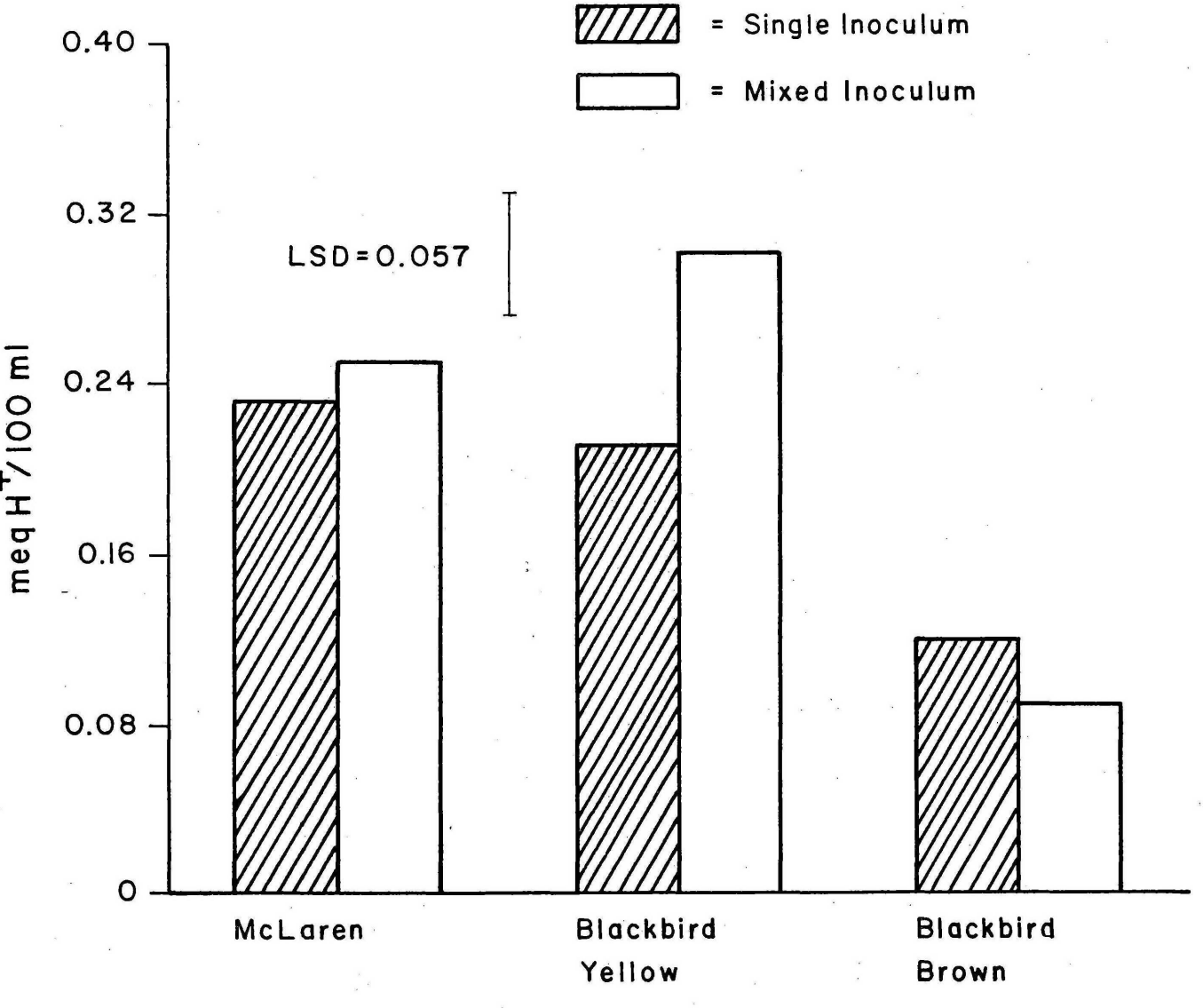

Figure 4. Acid produced in mine spoils after 2 weeks incubation, showing the interaction between spoil and inoculum type.

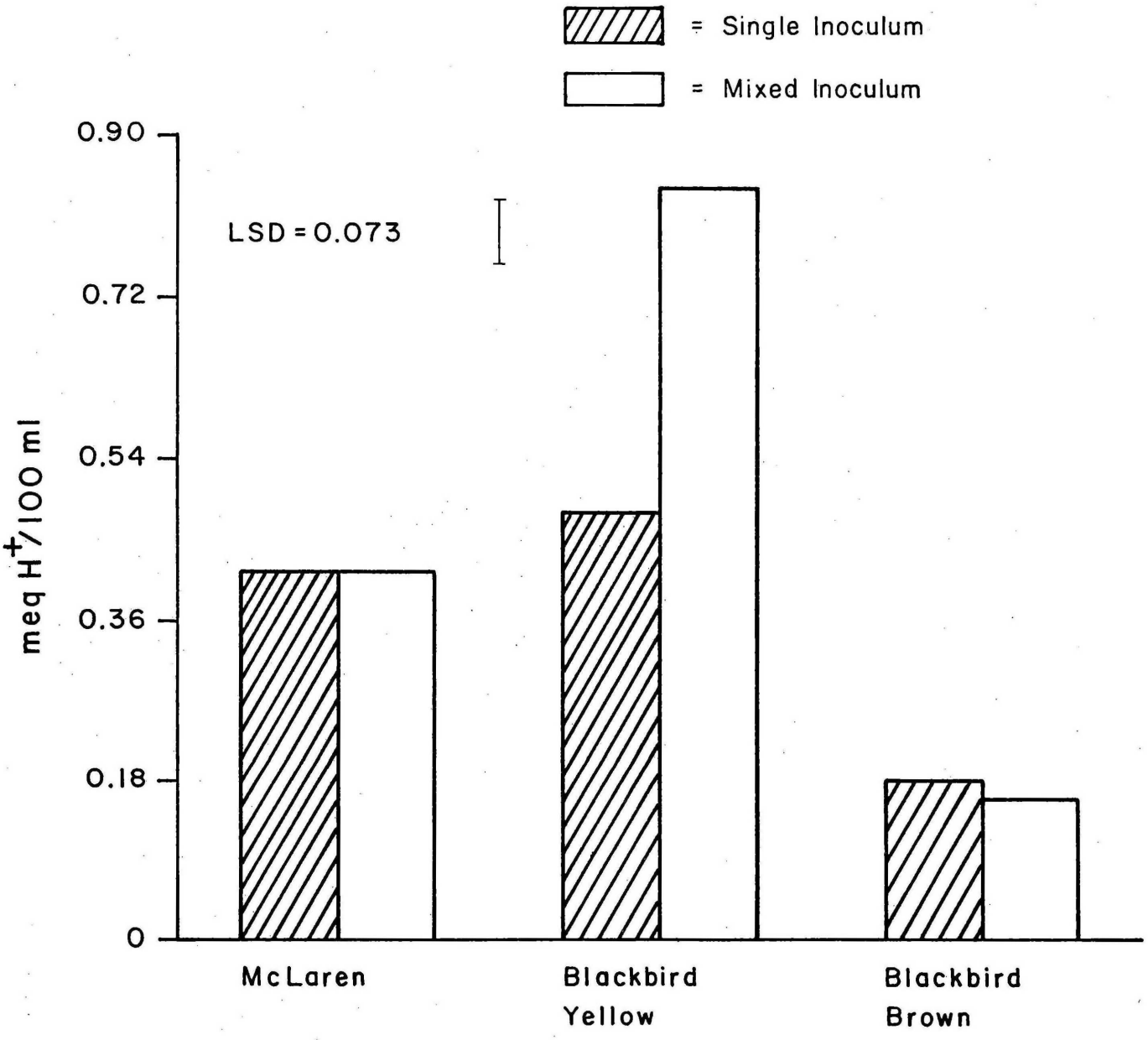

Figure 5. Acid produced in mine spoils after 6 weeks incubation, showing the interaction between spoil and inoculum type.

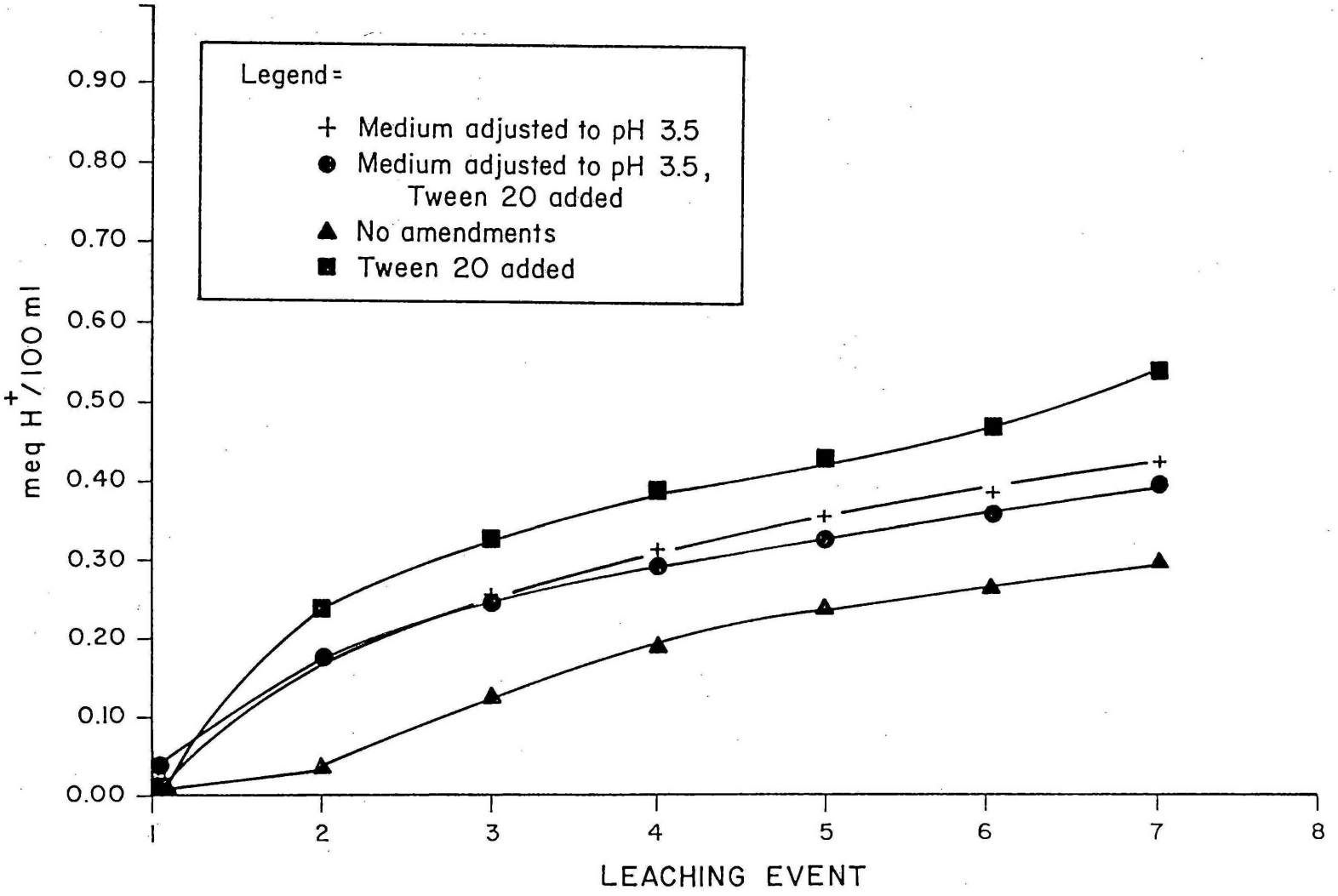

Figure 6. Cummulative acid production from the McLaren spoil inoculated with T. ferrooxidans alone.

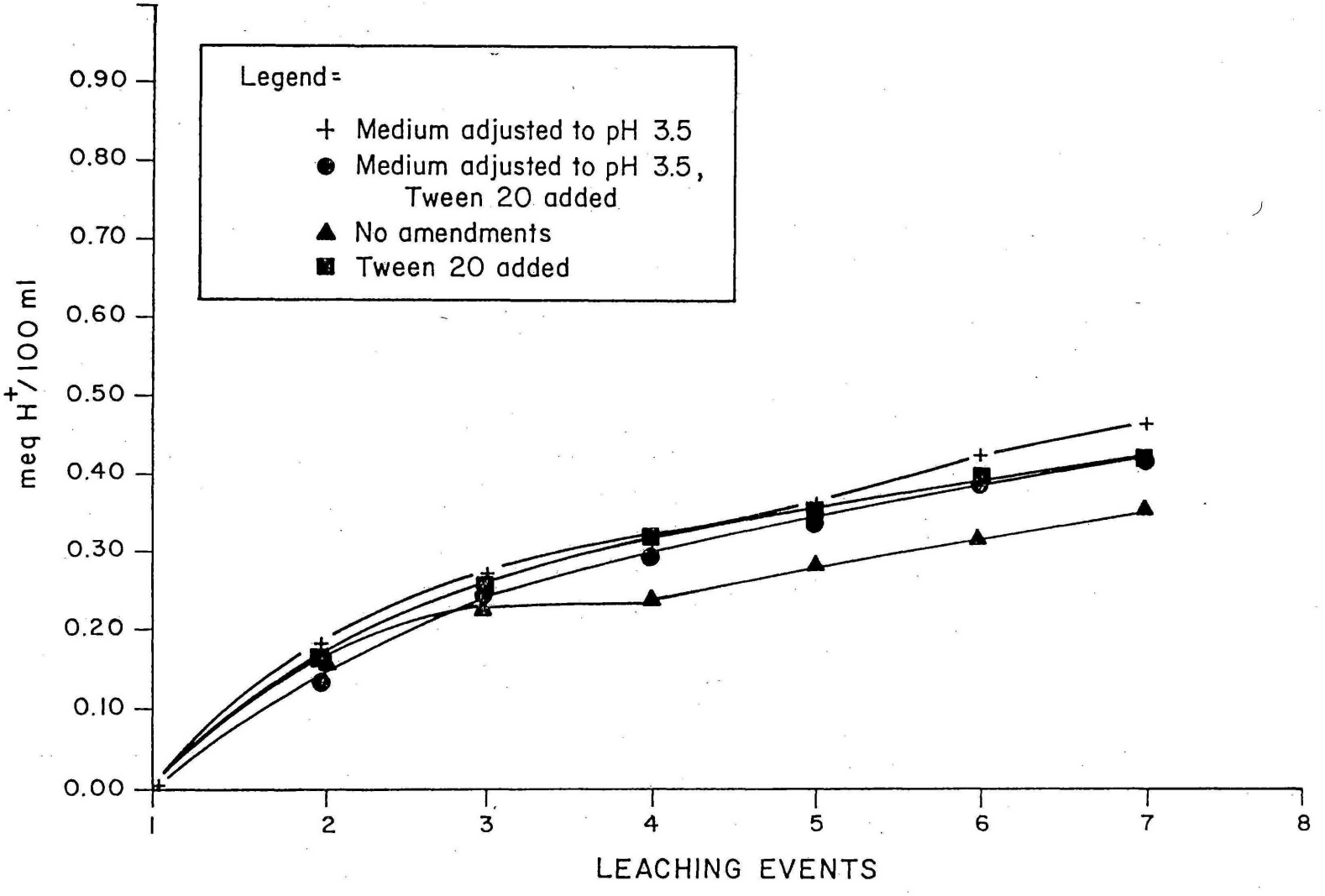

Figure 7. Cummulative acid production from the McLaren spoil inoculated with a mixture of Thiobacilli.

highest (Figure 3), but was surpassed in the second week by the Blackbird yellow sample inoculated with mixed Thiobacilli (Figure 4). This pattern continued through the sixth week of incubation (Figure 5). As with the Blackbird brown sample, no significant difference in acid production was observed from the type of inoculum added to this the McLaren spoil (Figures 4 and 5).

After the first incubation week (second leaching event), acid production in the Blackbird yellow sample was comparable to the McLaren sample, and was significantly higher than the Blackbird brown sample, but no significant effects of any treatment were observed (Figure 3). By the end of the second week of incubation, however, acid production in the Blackbird yellow sample inoculated with a mixture of T. ferrooxidans, T. thiooxidans, and T. neapolitanus was significantly higher than in the sample inoculated with T. ferrooxidans alone, and had surpassed acid production in the McLaren samples (Figure 4). The rate of acid production in the Blackbird yellow sample with mixed inoculum continued to be highest through 6 weeks of incubation (Figures 5, 8, and 9). Acid production in the Blackbird yellow mixed inoculum treatments was essentially linear over the 6 weeks incubation period (Figure 9) indicating that available reduced sulfur remained in ample supply. Figure 8 shows the acceleration in acid production rates observed in the Blackbird yellow single inoculum treatments near the end of the incubation period. This suggests that a population of Thiobacilli more adept at sulfur oxidation was developing. Extended incubation may show acid production rates in this treatment accelerating to be equivalent to the mixed inoculum treatments.

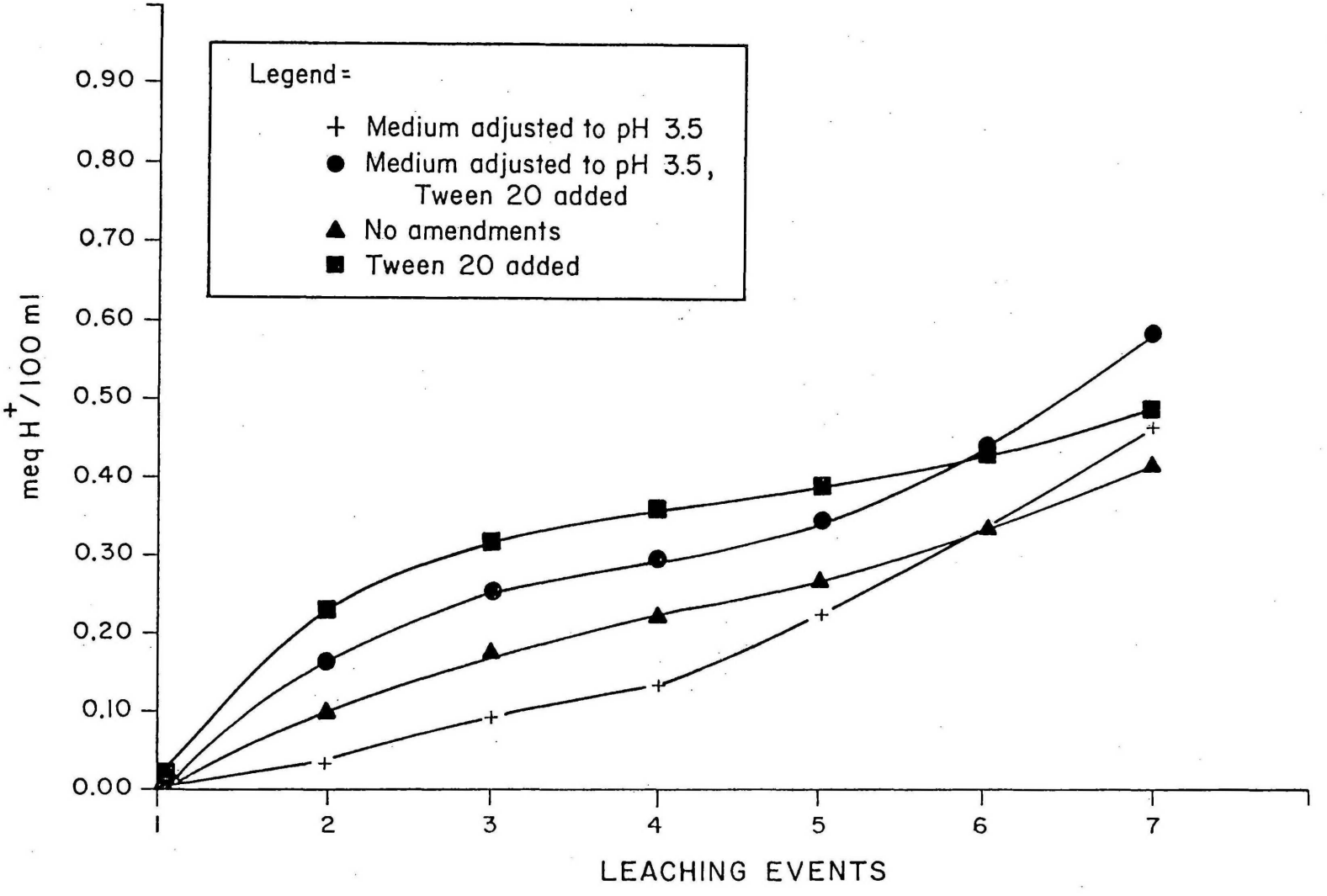

Figure 8. Cummulative acid production from the Blackbird yellow spoil inoculated with T. ferrooxidans alone.

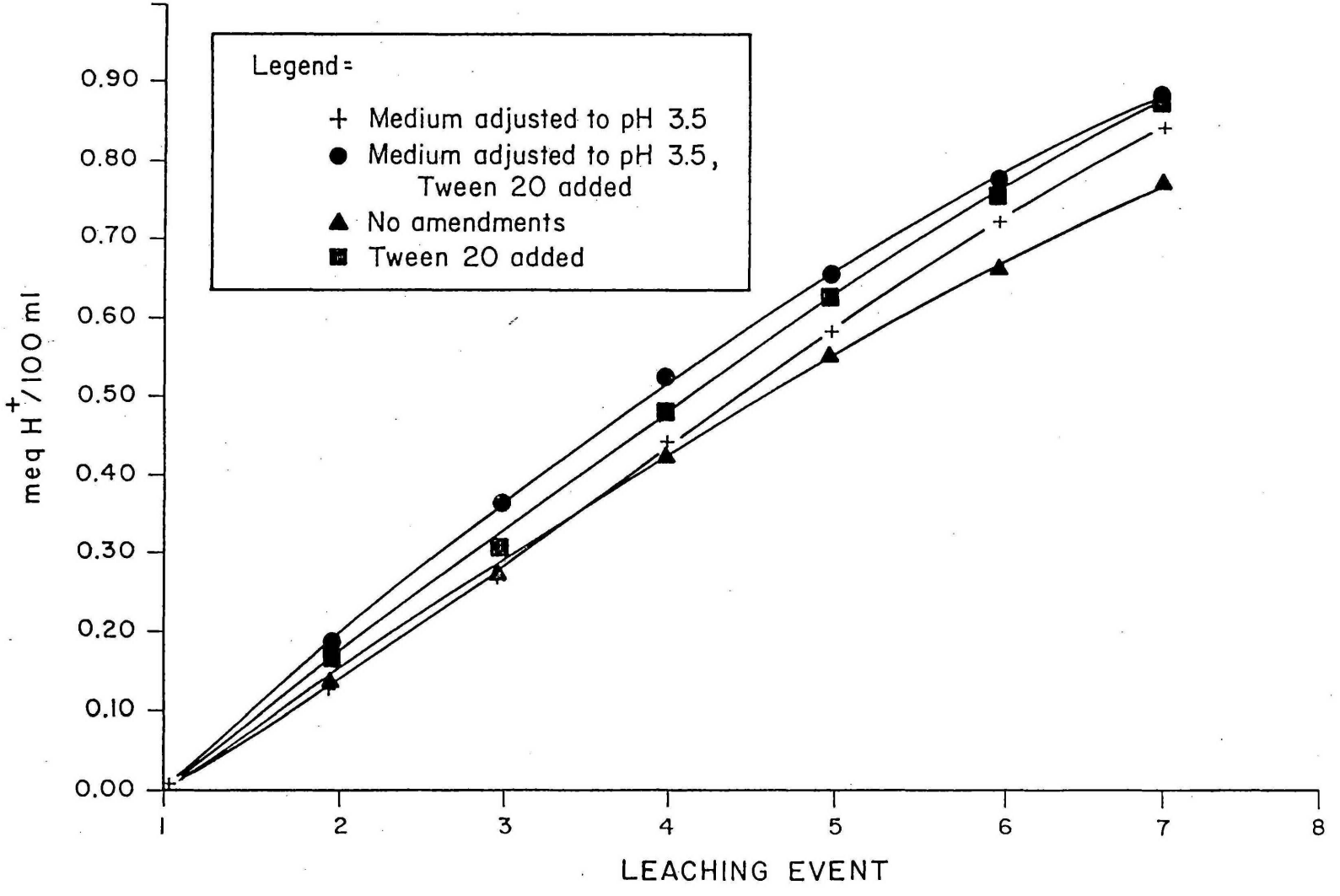

Figure 9. Cummulative acid production from the Blackbird yellow spoil inoculated with a mixture of Thiobacilli.

Acid production, averaged over all spoil samples, was significantly affected by the interaction between inoculation treatments and surfactant addition. The interactions effect continued from the first week through the third week of incubation. Figure 10 shows that in the first week there was significantly more acid production in single inoculum treatments when surfactant was added. In the first through the third week, there was no significant effect of surfactant on mixed inoculum treatments, but mixed inoculum treatments produced significantly more acid than single inoculum treatments without surfactant (Figures 10 and 11). In the third week there was no significant difference between acid production in the mixed inoculum treatments and the single inoculum treatments with surfactant (Figure 11). After 6 weeks incubation the interaction between inoculation and surfactant treatments was insignificant, but a significant enhancement of acid production by surfactant treatment, averaged over all other treatments and spoils, was observed (Figure 12).

Adjustment of the nutrient medium to pH 3.5 prior to application did not affect acid production except in an interaction with surfactant addition in the fourth week (Figure 13). At that time, treatments without pH adjustment and without surfactant produced significantly less acid than the non-pH adjusted treatments with surfactant, or the pH adjusted treatment with and without surfactant.

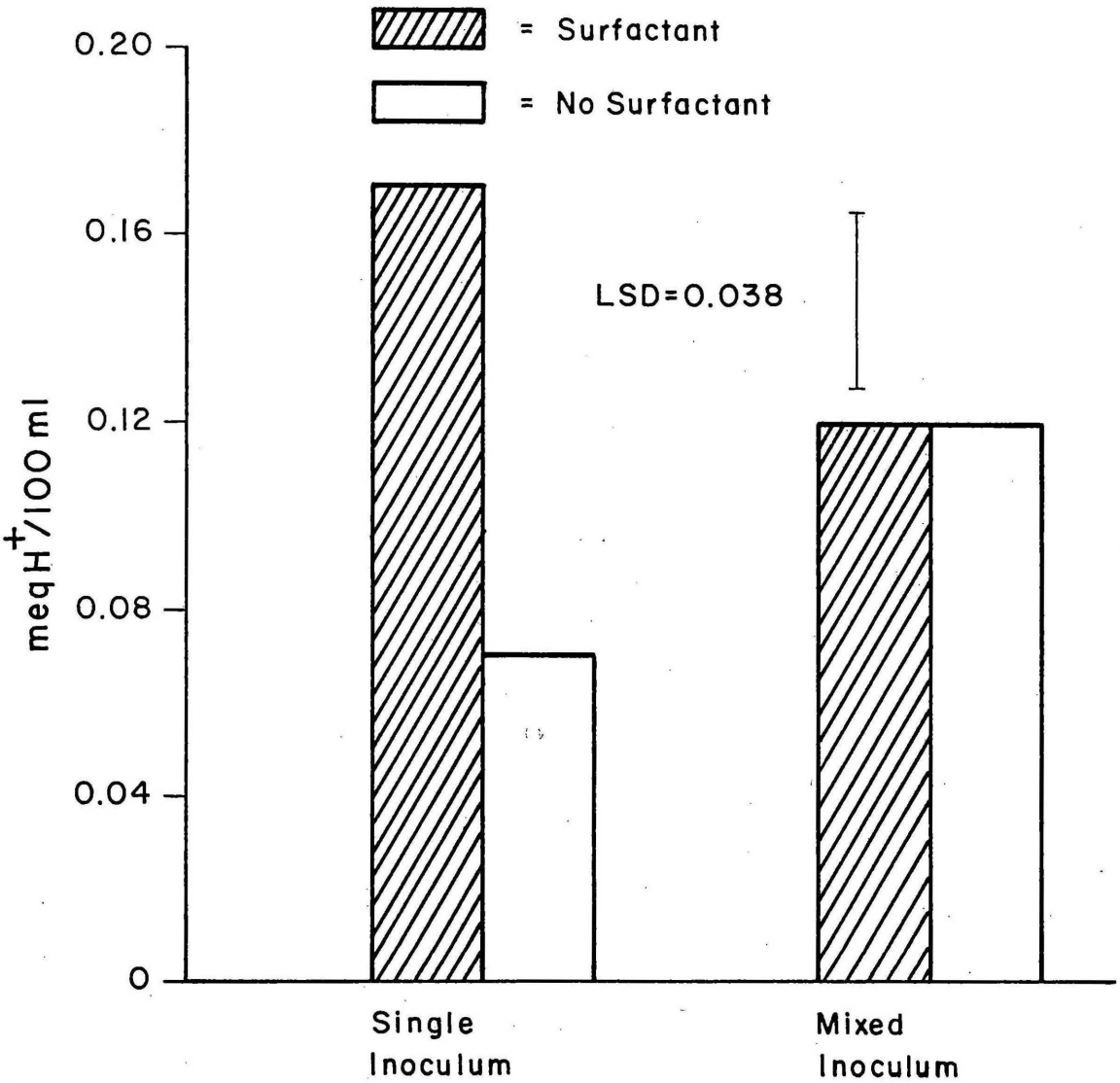

Figure 10. Average acid production in the Blackbird brown, Blackbird yellow, and McLaren spoils after 1 week incubation showing the interaction between inoculum type and surfactant addition.

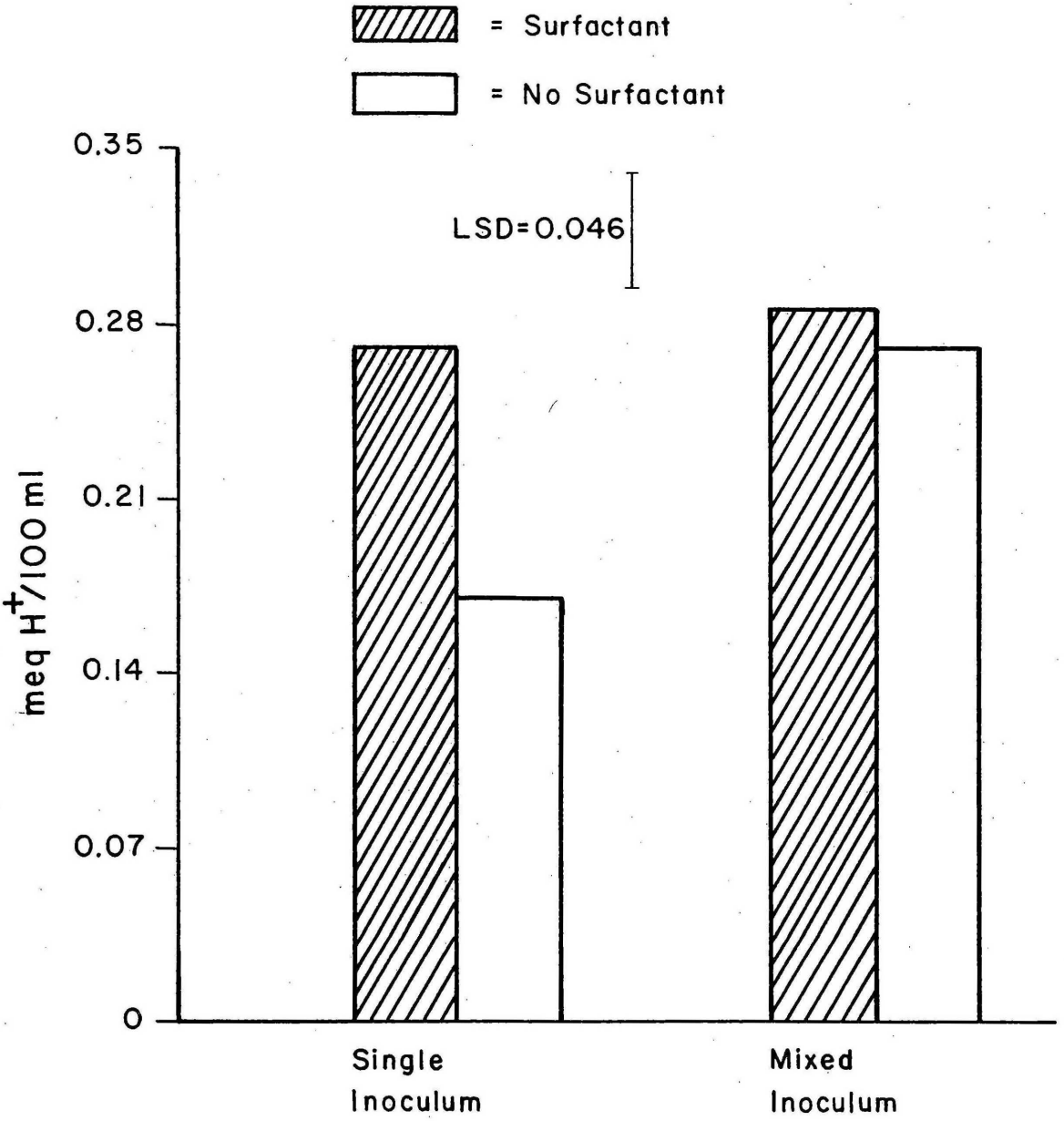

Figure 11. Average acid production in the Blackbird brown, Blackbird yellow, and McLaren spoils after 3 weeks incubation showing the interaction between inoculum type and surfactant addition.

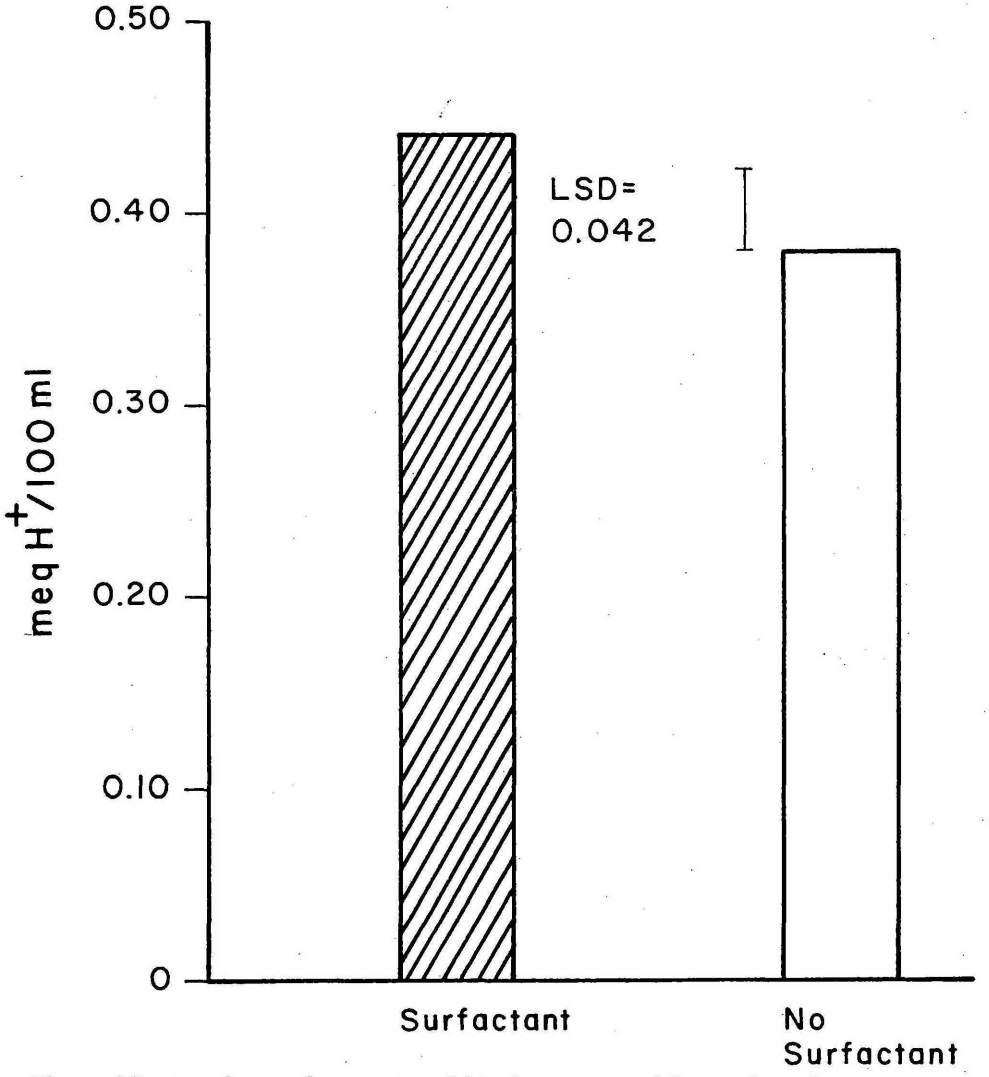

Figure 12. The effect of surfactant addition on acid production average across the Blackbird brown, Blackbird yellow, and McLaren spoils.

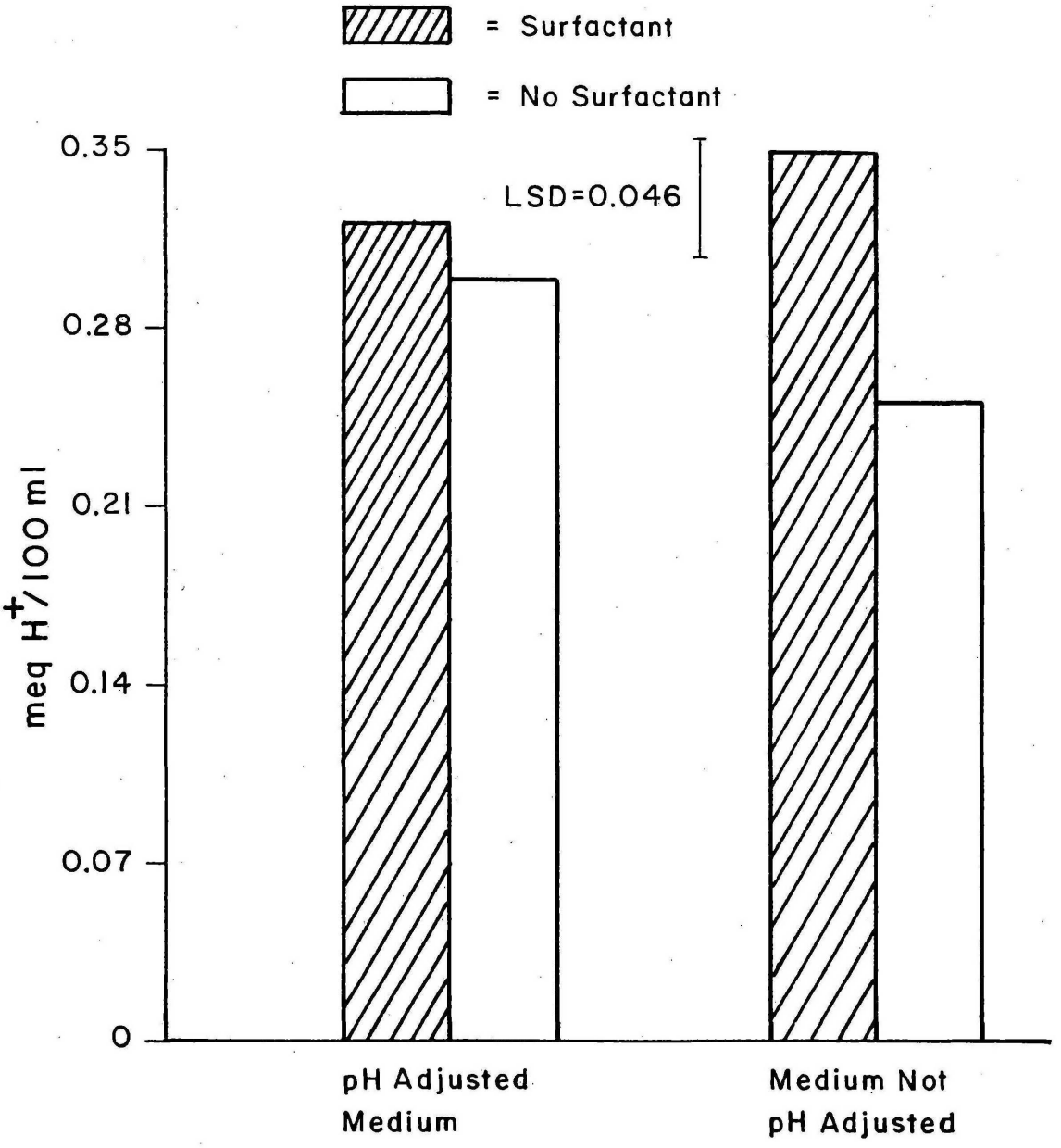

Figure 13. Average acid production after 4 weeks incubation, in the Blackbird brown, Blackbird yellow, and McLaren spoils showing the interaction between pH adjustment of the nutrient medium and surfactant addition.

CONCLUSIONS

These results indicate that the leaching bioassay is more sensitive to acid producing potential of mineral mine spoils than the closed culture bioassay. In certain spoils (e.g., the Blackbird yellow spoil) inoculation with T. ferrooxidans, T. thiooxidans, and T. neapolitanus enhances the sensitivity of the leaching test over inoculation with T. ferrooxidans alone. In general, the addition of 0.001% of the surfactant Tween 20 enhanced the sensitivity of the leaching test; especially early in the assay where inoculation with T. ferrooxidans alone was used. The importance of adjusting the pH of the nutrient medium to 3.5 appears marginal and should probably not be included as a routine part of the leaching assay. Initial indications are that where mixed inoculation is used, the general pattern and the relative intensity of acid formation can be estimated after 4 weeks of incubation (five leachings).

TENTATIVE BIOASSAY FOR ACID PRODUCTION POTENTIAL IN MINERAL MINE SPOILS

General

Periodic leaching of a 15 g sample of finely ground spoil is used to remove acid produced under optimized nutrient and incubation conditions. The acid produced is titrated and the cumulative acid production is recorded. A minimum of 4 weeks incubation and leaching (five leaching events) is recommended.

Apparatus

1. Incubation/leaching cup: A common disposable plastic 8 to 10 oz drinking cup with several 1/8 inch holes drilled in the bottom may be used. Cups can be supported in the mouth of a narrow mouth mason jar to receive leachate.

2. Glass wool: Medium coarseness.

3. HCl washed quartz sand or vermiculite: Wash sand in three volumes of 6 N HCl and rinse with water until the rinse water is near neutrality.

Reagents

1. Leaching solution: 0.01 M $CaCl_2$.

2. Nutrient solution: 3 g $(NH_4)_2SO_4$, 0.1 g KCl, 0.50 g, 0.50 g $MgSO_4 \cdot 7H_2O$, 0.01 g $Ca(NO_3)_2$, and 0.01 g Tween 20; diluted to 1 liter.

3. Titrant: 0.25 N NaOH.

Procedure

1. Set-up:
   a. Blend 15 g spoil with 15 g acid washed sand or an equal volume of vermiculite.
   b. Cover the bottom of the incubation/leaching cup with a pad of glass wool, add the spoil sand/vermiculite mixture, and cover the spoil mixture with another thin pad of glass wool. This prevents disturbance of the spoil mixture during the leaching process.
   c. Prepare blanks with all ingredients except soil, including 15 g of sand.
   d. After leaching, cover the cups with parafilm to diminish moisture loss to evaporation. The bottom holes are left open to allow gas exchange.
2. Leaching and nutrient addition:
   a. Leach the spoil initially with 150 ml of 0.01 M $CaCl_2$ followed by 25 ml of nutrient solution.
   b. Measure the volume of leachate collected after dripping from the bottom of the cup has ceased.
   c. Titrate the acidity of the leachate to pH 7.0 $\pm$ 0.2.
   d. Repeat the leaching and titration procedure weekly until acid production between leaching events begins to slow; then every two weeks until acid production becomes nil.
3. Calculation of results:
   a. Calculate acid production per gram of spoil at each leaching as follows:

meq H^+/ml leachate x X ml leachate/15 g = meq H^+/g

where X= total number of ml of leachate collected.

b. Cumulative acid production may be used to rank the acidification potential of the spoil.

RECOMMENDATIONS

1. Leaching bioassays need to be continued over a longer period of time to see when acid formation will cease in the Blackbird yellow and McLaren spoils and to determine the fraction of total and non-acid extractable sulfur oxidized in the bioassay.

2. The ability of the bioassay to predict and rank or quantify acid production needs to be verified in lysimeter tests under field conditions. These lysimeter tests should be continued through at least one full year so that weathering effects will be included in the results.

3. The relationship between bioassay results and chemical characteristics of the spoil needs to be examined to determine if a reliable, chemically determined predictor of acid formation potential can be found. For example, an examination of the data in Table 1 and the leaching bioassay results reveals that bioassay acid production was nil in samples that have a ratio of soluble and exchangeable sulfur to acid extractable sulfur greater than 0.3. This suggests that spoils with a tendency to form soluble sulfates are not likely to produce acid even when the sulfur content is high and in excess of acid neutralization potential (e.g., Leviathan 2 and Six Rivers).

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